Influence in time and space of non-crop elements with associated functional traits on biocontrol, within the Montepaldi Long-Term Experiment, Tuscany *

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Summary

Highly intensive practices of agriculture and an irrational usage of pesticides have resulted in environmental sources exploitation and damaging of surrounding habitats. In this scenario, the role of non-crop elements such as weeds and semi-natural habitats is crucial for enhancing natural enemy abundance and therefore supporting biocontrol effect. The aim of the research was to evaluate the correlation between presence of arthropods and plant species composing non-crop elements. Biocontrol was evaluated depending on: (1) distances from field margins, (2) management of the system and (3) period of sampling. An analysis of plant, with associated functional traits, and arthropods (4) was performed to identify which plant species likely support predators. Main findings highlighted an overall similarity between samples collected in different periods and spots. This is likely explained by the relatively small size of the experimental site with resulting strong interactions among all the systems and a strong influence of the surrounding landscape on arthropod biodiversity. However, the biocontrol effect was found to be high even in the farther spots from the margins, in every system and over the whole sampling period. In conclusion, *Cirsium arvensis* resulted to be the plant species associated with a higher abundance of predators.

Quote of the article: Gaifami-Pacini-2017-Montepaldi

Introduction

Beside the main role of agriculture in providing sources for humans, many ecosystem services such as biocontrol are attributed to agriculture (Power, 2010). However, ecosystem services provided by natural enemies are under pressure. The direct effect of an over use of pesticides is a dramatic increase of arthropod resistance to pesticides as well as unfavorable effects on

potential natural enemies (Perrings et al., 2006). In this scenario, the biocontrol made by beneficial organisms acquires a fundamental importance, since its role is crucial in both reducing the pest number and decreasing the pesticide usage. In order to preserve beneficial organisms, the role of elements within the field (weeds) and at the field margins (semi-natural habitats) is significant. Even though weeds and semi-natural habitats are usually negatively perceived by farmers as they consider them a threat for the main crop. Weeds compete with the main crop for nutrients, light and water, while semi-natural habitat reduce the remunerative land of the farm (Moonen et al. 2008). However, these elements may provide several benefits to natural enemies including nectar, pollen, shelter and overwintering habitat (Tscharntke et al., 2005, Balzan et al., 2014). Indeed, the majority of arthropods need nectar and sometimes pollen to integrate their diet in adult stage (Arnett et al., 2002).

Plant traits of non-crop vegetation, such as weeds and semi-natural habitats components, may significantly affect source availability and accessibility to arthropods and other flower visitors. The nectar accessibility depends on floral organ display and morphology. In particular, depth and width of nectar-protecting structure along with the length of the arthropod proboscis significantly affect flower resource accessibility (van Rijn et al., 2015). A wider range of flowers included in the system leads to a higher resources abundance throughout the growing season, along with supporting a higher diversity of biocontrol species. Accordingly, in periods when a higher number of flowers bloom, more abundant resources will be provided (Mohrmann, 2015).

In conclusion, an appropriate management of non-crop elements is crucial for supporting beneficial organisms throughout the growing season and the ecosystem services they provide (van Rijn et al., 2015).

Materials and Methods

In this study we evaluated the effects on predation rate (PR) and arthropod abundance (AA) depending on variation in space, production method and time. For this purpose, three *a priori* defined factors were selected: (1) *Distance* from the margin, (2) *System* of sampling (under different managements) and (3) *Period* of sampling. In addition, the analysis of arthropod abundance was put in relation with the presence of *Plants* with associated functional traits (4). Two methods were applied to evaluate the AA and consequently biocontrol (BC). The first consists in counting visually the arthropods lying within a metal frame. The second consists in beating the plants with a stick and identifying the arthropods falling into a tray. Aphids were the selected pests of the analysis. The PR was measured through exposing sentinel cards baited with *Ephestia kuehniella* eggs. Plant species with corresponding functional traits were identified within the barley field and adjacent semi-natural habitats (4). This methodology was applied to the Montepaldi Long Term Experiment (MoLTE, http://www.dispaa.unifi.it/vp-463-molte.html?newlang=eng) of the University of Florence, situated in the municipality of S. Casciano Val di Pesa, Tuscany. The PR and the AA evaluation was conducted on three barley fields belonging to corresponding micro agro-ecosystems managed with different methods: (a) “Old Organic” (OO), organic since 1991; (b) “New Organic” (NO), organic since 2001, integrated between 1991 and 2000; (c) “Conventional” (CO). Observations were carried out considering fields and surrounding semi-natural habitats (hedgerows and flower strip). Both PR and AA were evaluated within the margins, at 10m, 20m, 30m from the margins, in three systems (OO, NO, CO) and in April, May and June.

Two different statistical methodologies were applied. In order to analyze the AA as well as the relationship between flora and arthropods, a *multivariate* analysis was performed. The PR was evaluated by a *binomial regression* analysis. For the multivariate analysis, the software Primer 6 - Version 6.1. was used, while the binomial analysis was performed by the software R Studio - Version 0.99.902.

Binomial distribution- In binomial distributions, the probability is referred to each egg: 0 indicates a retrieved egg (non-predated), while 1 corresponds to predated, after the exposure time. Boxplots were generated in order to graphically show the results. Furthermore, a Generalized Linear Model (GLM) was performed with R-Studio. GLM is a generalization of ordinary linear regression which allows to analyze the correlation between all the variables (distance, period, system).

Multivariate analysis- The multivariate analysis included an analysis of similarities (ANOSIM) between two groups of samples generated by fixed factors defined *a priori* (distance, period, system). The degree of separation is expressed by a useful comparative statistical test (R) whose absolute value lies between 0 (indicating similar couples) and 1 (for dissimilar couples). Whether the overall values of R will be close to zero, it means that *a priori* defined factors cannot be considered as a main driver of separation among groups. Therefore, it was necessary to create new groups based on within-group similarity of arthropods species. In order to distinguish the new groups, a multi-dimensional scaling plot (a statistical ordination technique supplying a representation of samples in a two or three-dimensional graph) was inspected in combination with a corresponding Cluster Analysis (CA) dendrogram. Variables which contributed the most to form the groups were emphasized to characterize each group of samples. By evaluating the dominant variables of each group, it was possible to understand which factors influenced the arthropod abundance.

In order to analyze arthropod and plant correlations, we analyzed which were the plant species characterizing the groups of plots previously identified based on arthropod within-group similarity. This allowed to cross-check data found during arthropod and plant sampling. Following, for each plant found in the field in the three months of experiment, its blooming period and nectar accessibility were considered. Therefore, each frequency of sampling was weighted by a *Nectar Accessibility Score* - based on the corolla diameter and the nectar depth - for each specific blooming period of that species. As for the arthropod analysis, a prevalent percentage of plant species distinguished each group of variables.

Results and Discussion

Binomial distribution- The main results of the binomial regression, showed that egg predation rate did not significantly differ depending on factors defined *a priori* (distance, period, system). As Fig. 1 shows, although there are some overall slight differences (such as a higher PR in May compared to April), there is also a wide range of variability among samples.

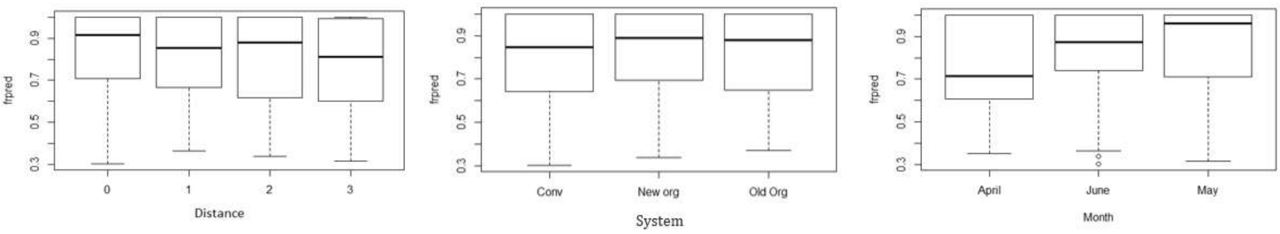

Fig. 1: Boxplots illustrating the fraction of egg predation (y-axis) plotted against the a priori defined factors (x-axis). Points more than 1.5 times the interquartile range above the third quartile and points more than 1.5 times the interquartile range below the first quartile are defined as outliers and plotted individually (Crawley, 2007).

These findings were in line with the outcome of the GLM, further confirming that the PR significantly depended on the interaction between all the factors, and not only on one.

Therefore, we can conclude that similar PR among the samples is likely due to the perception of similar habitats for predators. Possible explanations were addressed to explain similarities in time and space. The non-use of insecticides even in the system managed under conventional practices makes the habitats similar for both pest and predators; relatively short distances within the sampling systems (maximum 30m from the margins) and between the systems (roughly 175m) did not affect arthropod abundance, which likely migrated from one system to another one. In addition, the landscape surrounding the experimental site includes many

ecological infrastructures, wood and shrub areas and small scale fields, making the habitat very diversified and complex (Fig. 2).
This helps to create an environment favorable for enhancing and supporting natural enemy abundance, with resulting migration in the experimental field. However, this general similarity between samples indicates a positive action by predators over the whole period, even far from the margins, and in the conventional system.

Fig. 2: Landscape view of Montepaldi Long Term Experiment (MoLTE). Semi-natural habitats contribute to the beauty of the landscape and biodiversity of the experimental site .

Multivariate analysis- ANOSIM results highlighted an overall similarity between samples (almost all the R values of the groups defined *a priori* close to 0). The only difference was recorded in June (R~0.6) which significantly differed from April and May. This was due to the higher number of predators along with a lower number of aphids recorded.
The overall resemblance between samples indicates that groups defined a priori were not decisive for affecting the AA. Therefore, new groups were generated by inspecting the MDS plot (Fig. 3) and the CA dendrogram.

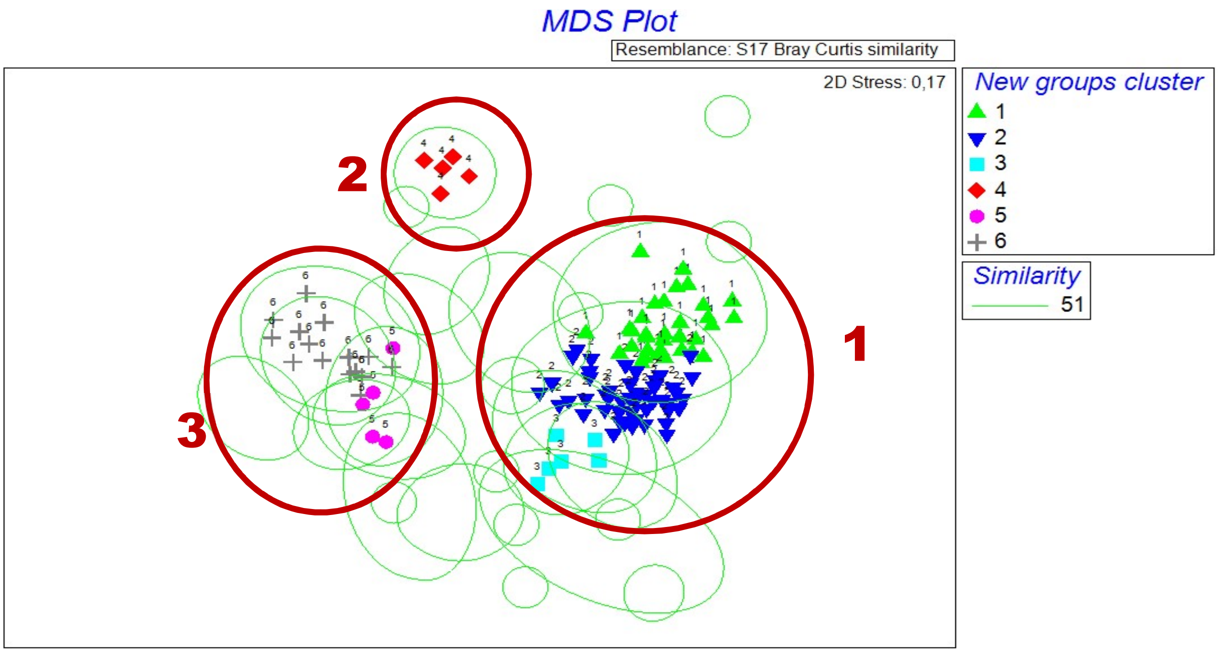

Fig. 3: Two-dimensional MSD Plot based on the Bray-Curtis resemblance matrix with graphical division into six new groups with a similarity level of 51%. Groups were obtained by superimposition of results of cluster analysis on the multi-dimensional scaling plot representing arthropods observations at the Montepaldi Long Term Experiment (MOLTE), Florence, Tuscany. The stress value of the

representation is 0.17, indicating a useful two-dimensional picture. Results were obtained after standardization by percentage of the variables and calculation of a similarity matrix based on the Bray–Curtis coefficient. Each group is represented by a different symbol explained in the legend. Three main clouds of groups are indicated by red circles.

A total of six new groups of samples were generated based on a minimum level of within-group similarity between observations of 51%. In the MDS graph, three main clouds of samples are represented with red cyrcles, clearly separeted from each other, and composed respectively by groups 1-2-3 (Cloud 1), 4 (Cloud 2) and 5-6 (Cloud 3). Each cloud resulted to be characterized by the predominant contribution of: aphids (Cloud 1), beetles (Cloud 2) and predators (Cloud 3).

SIMPER analysis further confirmed and explained the graphical output of MDS plot: three clouds of samples are characterized by the predominant contribution of aphids, beetles and predators, respectively.

In order to provide a more general overview about how arthropod abundance was distributed in time and space, a summarizing table was created (Table 1).

Table 1: Percentages of composition (%) of the six groups created by the superimposition of MDS and Cluster Analysis, for each of the factors defined a priori (period, distance, system). Bold data highlight most relevant findings.

	Period			Distance				System		
	A	**M**	**J**	**0**	**1**	**2**	**3**	**OO**	**NO**	**CO**
Gr 1	**63.6**	30.3	6.1	24.2	30.3	21.2	24.2	63.6	12.1	24.2
Gr 2	34.0	**61.7**	4.3	19.1	27.7	27.7	25.5	40.4	19.1	40.4
Gr 3	**50.0**	**50.0**	0.0	0.0	50.0	50.0	0.0	0.0	50.0	50.0
Gr 4	0.0	0.0	**100.0**	**60.0**	**40.0**	0.0	0.0	**80.0**	0.0	20.0
Gr 5	0.0	0.0	**100.0**	0.0	40.0	60.0	0.0	100.0	0.0	0.0
Gr 6	0.0	0.0	**100.0**	18.8	18.8	50.0	12.5	25.0	37.5	37.5

Legend: A=April, M=May, J=June; 0= 0m, 10=m, 2=20m, 3=30m; OO=Old organic, NO=New organic, CO=Conventional.

Groups of samples characterized by the dominance of aphids (Cloud 1) were mostly recorded in April and May, while non-aphid groups (Cloud 2 and 3) in June. Beetles (Coleoptera, including notable amount of ladybirds, *Coccinella magnifica*), the dominant species in Cloud 2's samples, were found mostly in the Old Organic system and inside or adjacent to the margins.

Similarities between groups explained by pest presence are characterized by the presence of *Convolvulus arvensis* and *Anthemis arvensis*. The large contribution of these two species is due to the high frequency of plants recorded, a high NA score and an extended blooming period. While Coleoptera seem to be characterized mainly by the presence of *Trifolium alexandrinum*, on the other hand, predator groups (Cloud 3) were mainly characterized by *Cirsium arvense*, *Convolvulus arvensis* and *Anthemis arvensis*. Therefore, it is not possible to conclude whether *Anthemis arvensis* or *Convolvulus arvensis* support the presence of pests or predators, as they are associated with both of them. However, we can assume that the presence of *Cirsium arvense* will support more likely predators than pests.

Conclusion

We cannot make conclusive claims whether PR or AA were influenced by non-crop elements because there are not reference systems without these elements. However, we can certainly conclude that in all the systems, predators occurred largely and constantly in time and space, even in the farther spots from the margins, and over the whole sampling period. Significant overall similarities between arthropod assemblages is likely explained by relatively similar habitats and strong interaction among all the organic and conventional systems. The former was due to lack of insecticide use in the CO field as well as similar plant composition of semi-natural habitats and weeds. The latter can be attributed to the fact that relatively short distances of observation points within fields and of observation points between barley fields belonging to different OO, NO and CO micro-agroecosystems facilitated the mobility of arthropods among samples and contextually decreased the potential differences. Therefore, it is hard to establish which management or distance from the margin or even which period will be more beneficial for predators, enhancing BC.

Regarding the semi-natural habitat composition, when BC is to be implemented, it is fundamental to maintain and support those plants with high nectar accessibility score throughout the whole year, aiming to support predator, without benefiting pests. *Cirsium arvensis,* for instance, may be one of them since it resulted more likely that it supports predators than pests. Further researches may deeper investigate the reason why this species is associated with predator abundance.

Acknowledgement

The funding provided by the H2020 ERA-NET CoreOrganic+ FertilCrop project is gratefully acknowledged. This research has been performed as master thesis of the first Author, under the master in Organic Agriculture at Wageningen University. It was the result of a fruitful collaboration between the Farming System Ecology group of Wageningen University and the Department of Agrifood Production and Environmental Sciences, University of Florence (UNIFI-DISPAA), Italy.

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